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Therapeutic IgE antibodies: harnessing a macrophage-mediated immune surveillance mechanism against cancer

Running title: IgE activates macrophages against tumors

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Abstract (193 words)

IgG monoclonal antibodies have made significant contributions to cancer therapy, but suffer from several limitations that restrict their effectiveness in unleashing host immune system components against tumors. The development of monoclonal antibodies of an alternative class, namely IgE, may offer enhanced immune surveillance and superior effector cell potency against cancer cells. In our recent manuscript, we elaborate our proof-of-concept studies of a mouse/human chimeric IgE antibody (hMOv18 IgE), which is specific for the cancer-associated antigen folate receptor alpha (FR α). We demonstrate superior anti-tumor efficacy for IgE compared with an otherwise identical IgG in a syngeneic immunocompetent animal, and we identify TNF α /MCP-1 signaling as an IgE-mediated mechanism of monocyte and macrophage activation and recruitment to tumors. These findings draw parallels with powerful macrophage-activating functions employed by IgE against parasites, rather than allergic IgE mechanisms. The potential clinical application of IgE-derived drugs in clinical oncology is clear if the anti-tumor activity of MOv18 IgE in these preclinical experiments can be replicated in patients. In particular, different IgE antibodies with specificity for many other antigens already validated as targets for IgG suggest a wide potential for development of a novel class of antibody therapy.

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Antibodies in cancer therapy and their limitations

Immunotherapy has become one of the most dynamic and rapidly-expanding areas of cancer therapy in recent years. One arm of the immunotherapeutic armamentarium, namely monoclonal antibodies, has been used with considerable success over the past 20 years, and now constitutes a vital component of contemporary cancer therapy (1). Monoclonal antibodies in current clinical use variously induce their therapeutic effects either through molecular signal blockade (for example by competitive inhibition of ligand binding to a transmembrane receptor), or by recruiting effector cells expressing the Fc-gamma receptor family members. Despite impressive results, however, antibody-based treatments continue to face limitations. Adequate binding of antibody to tumor antigens depends upon favorable pharmacokinetics, and efficient penetration and retention of the molecule in the targeted tissue. These binding properties are determined by antibody size, shape, receptor affinity and valency. All currently approved antibodies are members of the IgG class, characterized by their large molecular size, very long serum half-life of up to 3 weeks, and poor tissue retention. As a result of these biological properties, IgG molecules do not provide very efficient surveillance of the tissue compartment, which may limit the overall efficacy of existing therapeutic antibodies.

Once bound to cell surface antigens, therapeutic antibodies have the potential to elicit immune-mediated tumor cell death, either by engaging cell lineages of the innate immune system, or by activating the complement cascade. Antibody-dependent cell mediated cytotoxicity and phagocytosis (ADCC and ADCP) are dependent on interactions between Fc receptors (FcRs) expressed on the surface of immune cells, and the antibody Fc domain. Indeed, Fc-mediated mechanisms of immune system engagement appear to play an important role in the anti-tumor efficacy of the majority of approved antibodies for cancer therapy today. However, the triggering of Fc-mediated immune effector cell engagement by therapeutic IgGs may be limited by several factors, including i) the low affinity of IgG for its Fc γ Rs, (requiring the formation of immune complexes to achieve adequate retention and Fc signaling of antibodies by effector cells), ii) glycosylation of antibody Fc regions (known to reduce Fc γ R affinity), iii) competition with native IgGs (especially IgG4) for

binding to FcγRs, and iv) inhibitory Fc receptors such as FcγRIIb expressed on B cells, macrophages, dendritic cells and neutrophils (2).

Macrophages in the tumor microenvironment

Cancer cells rely on resident and recruited 'accessory cells' to support their proliferation. Such cells include those forming the vasculature and lymphatics, tissue-specific mesenchymal support cells, and myeloid and lymphoid-lineage immune cells. Interactions between neoplastic cells and cellular components of the tumor microenvironment (TME) have prompted research into immunotherapeutic approaches aimed at neutralizing tumor-promoting chronic inflammation, or at releasing the cytotoxic activities of antigen-specific T cells (3).

Although the development of monoclonal antibody therapies has traditionally focused on targeting the tumor cell itself, more recently attention has shifted towards targeting the TME and its cellular components. Clinical application of IgG antibodies blocking inhibitory components of the immune checkpoint, in particular T cell signaling mediated by CTLA4 and PD-1, has been the most successful application of this new strategy to date (4). Reactivation or reprogramming of cells within the TME may provide the key to further success with antibody-mediated cancer immunotherapy.

Macrophages are a prominent component of the cellular TME, where they exert a profound influence over the tumor immunologic composition (5). Macrophages are phenotypically diverse members of the mononuclear phagocyte family, distributed throughout every organ of the body. Previously thought to be derived exclusively from circulating monocytes, tissue-resident macrophages have more recently been found to be established *in utero*, and to be able to replenish their numbers independently of circulating monocytes (6). These tissue-resident macrophages are maintained locally via colony stimulating factor 1 (CSF1), a key growth factor produced by the local tissue stroma (6, 7) and may also be enhanced by recruitment and differentiation of circulating monocytes (7). Tumor-associated macrophages (TAMs) are recruited into tumors following activation of their CSF1 receptor (CSF1R)

by either CSF1 or IL-34. In addition, the chemokine CCL2/MCP-1 may facilitate macrophage recruitment into tumors (8).

Under physiological conditions, macrophages act as phagocytes, serving as an early line of defense against pathogens. They are specialized to engulf and digest cellular debris and drive adaptive immune responses. Despite a common progenitor, TAM populations are functionally diverse, ranging from anti-tumor, pro-inflammatory (M1) macrophages to tumor-promoting, anti-inflammatory (M2) populations (5). Macrophages are therefore implicated both as essential mediators in anti-tumor immune responses as well as drivers of local tolerance and even tumor-promoting inflammation. Indeed, within the TME, innumerable tumor- and stromal-derived factors may suppress the tumoricidal activity of TAMs, endowing them with properties characteristic of M2 macrophages, so facilitating tumor growth, metastasis and immune evasion. In this way, TAM infiltration may correlate with poor prognosis and disease outcome in many human cancers (9). By contrast, dense tumor infiltration by macrophages in lymphoma patients is associated with improved outcome following a rituximab-containing regimen, in contrast to inferior survival in the absence of rituximab treatment (10). This suggests that tumor macrophage infiltration may be a favorable factor specifically in the context of treatment with monoclonal antibodies.

Enhancing the efficacy of antibody therapy: harnessing the effector functions of IgE antibodies

Efforts to boost the efficacy of anti-tumor IgGs have involved modification of the IgG constant (Fc) region to strengthen its ability to interact with the human immune system. Approaches to enhance this interaction include altering Fc region amino acid sequences (11) or changing the glycosylation pattern of the Fc region to enhance interaction with FcγRs on effector cells (12). Another strategy to optimize the antibody-immune system interaction has been exploration of antibodies with Fc regions of alternative immunoglobulin classes, such as IgE. Work in this area constitutes an important branch of the rapidly-growing field of AllergoOncology,

which aims to address the potential opportunities of IgE-mediated and Th2-biased cellular responses in malignant diseases (13, 14). The key hypothesis underlying IgE immunotherapy is that this antibody class can recruit a different effector cell population, utilizing the cognate FcεRs expressed on those cells. Innate immune cells such as macrophages may be reactivated and retargeted by anti-tumor IgE to overcome inhibitory effects of the TME.

Beside its critical role in allergy, IgE plays a physiological role in immunity against parasitic infections, by a number of different mechanisms and via a number of IgE receptor-expressing cell types including monocytes and macrophages (15). The particular properties that make IgE a key contributor to the allergic response, and permit protection against parasitic infections, suggest the potential value of antibodies in this class as therapeutic agents in cancer. The manifestations of local immune stimulation seen in parasitic infestation, with an ensuing cascade of effector cell activation and inflammation at the site of antigen provocation, might be harnessed by IgE therapies to induce tumor rejection. Macrophages are likely to be a key cell population implicated in such an IgE-mediated anti-cancer effect, because they are known to be pivotal effectors in the control of intracellular and extracellular parasites by IgE through engendering effector mechanisms such as ADCC and ADCP (16, 17).

The potential biological advantages of IgE antibodies outlined above, and the presence in solid tumors of many key FcεR-expressing immune effector cells including macrophages, provide a rationale for the development of tumor-specific therapeutic IgE molecules (13, 14). Work by Josephs et al. describes proof-of-concept studies building upon our previous studies of a mouse/human chimeric IgE antibody (MOv18 IgE) specific for the ovarian cancer-associated antigen folate receptor alpha (FRα) (13, 18-20). Here, in an immunocompetent rodent model of pulmonary metastases from a syngeneic tumor expressing human FRα, we demonstrated clear superiority of anti-tumor activity for IgE compared to IgG, in line with our previous findings in two *in vivo* models of cancer with reconstituted human cellular immunity (21).

A role for human monocytes was previously demonstrated *in vitro* via both ADCC (mediated by the IgE high affinity receptor FcεRI, expressed by a proportion of monocytes/macrophages), and ADCP (through CD23, the low affinity IgE receptor, expressed on the surface of IL-4-activated monocytes/macrophages). ADCC and ADCP are both known mechanisms of action for IgE in the defense against parasitic infections. This provided a rationale in favor of further exploring the IgE-mediated anti-tumor functions of these cells (19, 20). In a nude mouse xenograft model of FRα-positive patient-derived ovarian carcinoma with cellular immunity reconstituted using human peripheral blood mononuclear cells (PBMCs), control of tumor growth using chimeric mouse/human MOv18 IgE (hMOv18 IgE) was superior to that of the IgG1 anti-FRα counterpart. Tumor xenografts were infiltrated by human monocytes in hMOv18 IgE-treated mice. Use of monocyte-depleted PBMCs in this model resulted in a loss of the survival advantage conferred by hMOv18 IgE (20). This indicated that monocytes may play an important role in the anti-tumor effect of hMOv18 IgE *in vivo* (20).

Based on these findings, we sought to explore the role of the monocyte/macrophage lineage in mediating tumor cell killing by IgE in an immunocompetent model. We further hypothesized that IgE may recruit and re-educate these cells to adopt an activated phenotype.

IgE-mediated macrophage recruitment and activation

In the study recently reported in Cancer Research we sought to ascertain how anti-tumor IgE antibodies may be applied to recruit macrophages and to reprogram these cells to eradicate cancer cells (21). Our work reveals a previously-unappreciated contribution of a TNFα/MCP-1 cascade to monocyte and macrophage re-education and recruitment in an immunocompetent rat tumor model, specifically chosen for its suitability to examine IgE effector functions (Fig. 1).

MOv18 IgE inhibited tumor growth and induced pronounced infiltration of CD68-positive monocytes/macrophages deep into rat tumors. In concordance, the degree of intra-tumor macrophage influx significantly correlated with prolonged survival of

IgE-treated mice bearing patient-derived tumor xenografts. There is evidence to suggest that the location of TAMs in relation to tumor cells in human cancer can influence clinical outcome (22). Macrophage density within tumor islets was positively associated with survival of patients with lung cancer, whereas a concentration of these cells in the stroma, away from tumor cells, was negatively prognostic. Furthermore, the ratio of macrophage density in the tumor islets to macrophage density in the tumor stroma appeared to correlate better with survival (22). Therefore, effective MOv18 IgE-induced tumor infiltration by macrophages may represent a mechanistic explanation for the observed superior anti-cancer activity compared with IgG.

In the MOv18 IgE-treated immunocompetent model, the phenotype of tumor lung metastases-infiltrating macrophages is different to that of macrophages from the MOv18 IgG or buffer control groups: tumor-associated macrophages from MOv18 IgE-treated rats featured elevated surface expression of the macrophage maturation and co-stimulatory marker CD80, higher intracellular expression of the pro-inflammatory and cytotoxic mediator TNF α , and some elevation of IL-10. TNF α was also found to be a prominent cytokine within the airways of MOv18 IgE-treated rats (broncho-alveolar lavage – BAL), alongside the macrophage chemoattractant MCP-1 and IL-10. This points to the presence of a distinct macrophage compartment, and a specific immune mediator signature in tumor environments, both associated with IgE therapy.

In our study, we have shown that cross-linking of IgE, but not IgG, of any antigen specificity bound to monocytes triggers upregulation of TNF α expression (Fig. 1A). This is likely to be a function of the high affinity of IgE for Fc ϵ RI on macrophages, and may be facilitated by molecular patterns displayed by tumor antigens (designated Tumor-Associated Molecular Patterns; TAMPs) (23). TAMPs can promote crosslinking of IgE bound to Fc ϵ Rs on effector cells, thereby fostering more sustained interactions of macrophages with IgE than with IgG, and leading in turn to higher levels of TNF α in the tumor microenvironment. MOv18 IgE-mediated tumor ADCC was abrogated *in vitro* by TNF α receptor-specific blockade of monocyte effector cell functions,

pointing to the contribution of TNF α signaling in anti-tumor IgE effector functions (21).

In addition to TNF α , the other key analyte elevated in the BAL fluid from rats treated with MOv18 IgE was MCP-1, a member of the CC family of chemokines, a potent chemo-attractant for macrophages and a macrophage-related pro-inflammatory chemokine (24). We demonstrate that upregulation of TNF α by monocytes could promote enhanced MCP-1 expression by both monocytes and importantly by tumor cells (Fig. 1B). This IgE-specific macrophage activation and recruitment mechanism seems to be generalizable, since TNF α triggered higher MCP-1 production by a number of different tumor cell types.

Studies have suggested that MCP-1 produced by tumor cells may be responsible for chemotaxis of monocytes, mast cells and CD8 $^{+}$ T-cells into the TME (25). The markedly increased recruitment of macrophages deep into tumors observed in animals treated with MOv18 IgE, compared to MOv18 IgG, may therefore result from the MCP-1 upregulation described above (Fig. 1C).

Taken together, our findings are consistent with a positive feedback interaction engendered by IgE-, but not IgG-, Fc engagement and cross-linking on the surface of macrophages. Initial tumor cell–macrophage interactions fostered by tumor antigen-specific IgE may trigger a TNF α -mediated MCP-1 upregulation cascade that may further mobilize macrophages or recruit additional macrophages into tumors.

Re-programing macrophages towards a tumoricidal function

The plasticity of tumor-associated macrophages may be exploited therapeutically in order to restore their anti-tumor properties. Strategies to re-program Th2-driven myeloid cells to reduce the immunosuppressive status of macrophages, trigger anti-tumor immunity, or suppress tumor growth have all been pursued in several tissue-specific cancer models (26-30) (Fig. 1D). For instance, CSF1-neutralizing monoclonal antibodies and small-molecule CSF1R inhibitors have been evaluated, as monotherapy or in combination with chemotherapy, radiotherapy or other immunotherapies, for their ability to suppress macrophage survival and/or presence

in tumors (26, 31). TAMs have also been therapeutically manipulated by inhibiting the macrophage transmembrane receptor kinase RON (27). Other strategies include antibody-mediated activation of co-stimulatory CD40 or blocking of inhibitory IL-10, delivery of immunostimulatory cytokines such as IL-12, or the administration of Toll-like receptor (TLR) agonists including imiquimod (28-30). It may be that IgE therapies could join these other novel approaches in targeting pro-tumorigenic immune cells including macrophages, so altering the TME in a way that fosters their cytotoxic properties.

Although pivotal in the control of parasites via IgE, physiological macrophage activity appears to be suppressed in tumors. Our findings point to the possibility that administration of therapeutic IgE antibodies may re-direct macrophage functions, evolved to neutralize parasites, against cancer cells. TNF α , MCP-1, nitric oxide (NO) and IL-10 are all upregulated during parasiticidal activities of macrophages (17). Upregulation of TNF α and MCP-1 is detected in tumors in response to IgE therapy, but the classical Th2 cytokine IL-4 is notably absent. This indicates that parasite-targeting rather than allergic, mechanisms are dominant in this context. Furthermore, analysis of publically-available ovarian gene expression libraries suggests a positive prognostic role for elevated TNF α /MCP-1 levels, as well as for macrophage and Fc ϵ R markers, highlighting the clinical significance of this mediator signature in patients with cancer (21).

In the future it is possible that the clinical use of IgE class antibodies to combine engagement, recruitment and activation of tumor-associated macrophages could ignite the tumoricidal properties of these cells, and open new and compelling therapeutic opportunities. Improved understanding of relevant activating cytokine cascades, normally associated with parasite clearance, could further harness this hitherto unappreciated mechanism of human immune defense against cancer.

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Figure Legend

Fig 1. Effects of anti-cancer strategies including anti-tumor IgE antibodies on macrophages. Activation of macrophages by anti-tumor IgE mediates a $\text{TNF}\alpha/\text{MCP-1}$ axis (A-B) and promotes potent recruitment of macrophages (C). (A) IgE engagement with $\text{Fc}\epsilon$ receptors on the surface of macrophages is not sufficient for effector cell activation (i). Cross-linking of tumor-targeting IgE antibodies on macrophages by polyclonal anti-IgE (ii) or antigen-bearing tumor cells (iii) is necessary for upregulation of $\text{TNF}\alpha$ by macrophages. (B) $\text{TNF}\alpha$ then stimulates production of MCP-1 by macrophages and tumor cells, which promotes potent chemotaxis of further macrophages into tumors resulting in enhanced tumor cell-macrophage interactions and subsequent tumor cell death (C). Anti-tumor mechanisms of established and novel anti-cancer therapies on macrophages include: reprogramming to a new macrophage phenotype, macrophage recruitment, repolarization to an M1-like phenotype, activation to trigger antibody-mediated tumor cell death (e.g. by ADCC, ADCP, immunoactivatory cytokines), and suppression of immunosuppressive tumor-associated macrophage survival (D).

